

In the Sequence Listing:

Please insert the attached paper copy of the Sequence Listing as new pages 1 -179 in the above-captioned application. A computer readable (CRF) accompanies this response.

## AMENDMENTS

Please replace the paragraph beginning at page 6, line 17, with the following rewritten paragraph:

-- **Figure 1. 85P1B3 SSH sequence.** The 85P1B3 SSH sequence contains 259 bp. (SEQ ID. NO. : 724). The homology comparison in Figure 1 shows SEQ ID NOS 725-726, respectively, in order of appearance.

**AI**  
-- **Figure 2. The cDNA (SEQ ID. NO. :727) and amino acid sequence (SEQ ID. NO. :728) of 85P1B3.** The start methionine is underlined. The open reading frame extends from nucleic acid 13 to 702 including the stop codon.

**Figure 3. Amino acid sequence of 85P1B3 (SEQ ID. NO. :729).** The 85P1B3 protein has 229 amino acids.

**Figure 4. Sequence alignment of 85P1B3 (SEQ ID NO: 730) with GenBank accession number AAC39561.1 (AF025441), Opa-interacting protein OIP5 (SEQ ID. NO. :731).--**

Please replace the paragraph beginning at page 9, line 13, with the following rewritten paragraph:

**A2**  
--**Figure 21. Secondary structure and transmembrane prediction for 85P1B3. Panel A.** (SEQ ID NO: 729) The secondary structure of 85P1B3 protein was predicted using the HNN - Hierarchical Neural Network method (Guermeur, 1997, [http://pbil.ibcp.fr/cgi-bin/npsa\\_automat.pl?page=npsa\\_nn.html](http://pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_nn.html)), accessed from the ExPasy molecular biology server

*A2*  
*cont.*

(<http://www.expasy.ch/tools/>). This method indicates the presence and location of alpha helices (h), extended strands (e), and random coils (c) from the primary protein sequence. The percent of the protein in a given secondary structure is also given. Panel B. Schematic representation of the probability of existence of transmembrane regions of 85P1B3 based on the TMpred algorithm of Hofmann and Stoffel which utilizes TMBASE (K. Hofmann, W. Stoffel. TMBASE - A database of membrane spanning protein segments Biol. Chem. Hoppe-Seyler 374:166, 1993). Stretches of amino acids approximately 17-33 amino acids in length with a value greater than 0 are potential transmembrane helices. This program indicates the presence of one helix in 85P1B3. Panel C. Schematic representation of the probability of the existence of transmembrane regions and the extracellular and intracellular orientation of 85P1B3 based on the algorithm of Sonnhammer, von Heijne, and Krogh (Erik, L.L., et al., A hidden Markov model for predicting transmembrane helices in protein sequences. In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p 175-182 Ed J. Glasgow, et al., Menlo Park, CA: AAAI Press, 1998). This program indicates 85P1B3 to be an intracellular protein without transmembrane domains. These transmembrane prediction results are also summarized in Table XXV.--

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Please replace the paragraph beginning at page 17, line 17, with the following rewritten paragraph:

*A3*

- (a) a polynucleotide comprising or consisting of the sequence as shown in Figure 2 (SEQ ID NO.: 727), wherein T can also be U;
- (b) a polynucleotide comprising or consisting of the sequence as shown in Figure 2 (SEQ ID NO:727), from nucleotide residue number 13 through nucleotide residue number 699, wherein T can also be U;--

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Please replace the paragraph beginning at page 17, line 25, with the following rewritten paragraph:

*A4*

- (d) a polynucleotide that encodes an 85P1B3-related protein that is at least 90% homologous to the entire amino acid sequence shown in SEQ ID NO:728;

A4  
CON'T. (e) a polynucleotide that encodes an 85P1B3-related protein that is at least 90%

identical to the entire amino acid sequence shown in SEQ ID NO:728;--

Please replace the paragraph beginning at page 30, line 25, with the following rewritten paragraph:

--III.B.) **Expression of 85P1B3-related Proteins**

In an embodiment described in the examples that follow, 85P1B3 can be conveniently expressed in cells (such as 293T cells) transfected with a commercially available expression vector such as a CMV-driven expression vector encoding 85P1B3 with a C-terminal 6XHis (SEQ ID NO: 709) and MYC tag (pcDNA3.1/mycHIS, Invitrogen or Tag5, GenHunter Corporation, Nashville TN). The Tag5 vector provides an IgGK secretion signal that can be used to facilitate the production of a secreted 85P1B3 protein in transfected cells. The secreted HIS-tagged 85P1B3 in the culture media can be purified, e.g., using a nickel column using standard techniques.--

Please replace the paragraph beginning at page 71, line 15, with the following rewritten paragraph:

--Single chain antibodies comprise the variable domains of the heavy and light chain joined by a flexible linker polypeptide, and are expressed as a single polypeptide. Optionally, single chain antibodies are expressed as a single chain variable region fragment joined to the light chain constant region. Well-known intracellular trafficking signals are engineered into recombinant polynucleotide vectors encoding such single chain antibodies in order to precisely target the intrabody to the desired intracellular compartment. For example, intrabodies targeted to the endoplasmic reticulum (ER) are engineered to incorporate a leader peptide and, optionally, a C-terminal ER retention signal, such as the KDEL (SEQ ID NO: 708) amino acid motif. Intrabodies intended to exert activity in the nucleus are engineered to include a nuclear localization signal. Lipid moieties are joined to intrabodies in order to tether the intrabody to the cytosolic side of the plasma membrane. Intrabodies can also be targeted to exert function in the cytosol. For example, cytosolic intrabodies are used to sequester factors within the cytosol, thereby preventing them from being transported to their natural cellular destination.

Please replace the paragraph beginning at page 80, line 4, with the following rewritten paragraph:

--The chromosomal localization of 85P1B3 was also determined using the GeneBridge4 Human/Hamster radiation hybrid (RH) panel (Walter et al., 1994; Nature Genetics 7:22)(Research Genetics, Huntsville Al).

*A*  
The following PCR primers were used:

85P1B3.1 5' catgggactctgcatcttaattcc 3' (SEQ ID NO: 732)

85P1B3.2 5' caggttcaggcttattgctgtct 3' (SEQ ID NO: 733)--

Please replace the paragraph beginning at page 82, line 12, with the following rewritten paragraph:

*AS*  
--pGEX Constructs: To generate recombinant 85P1B3 proteins in bacteria that are fused to the Glutathione S-transferase (GST) protein, all or parts of the 85P1B3 cDNA protein coding sequence are fused to the GST gene by cloning into pGEX-6P-1 or any other GST- fusion vector of the pGEX family (Amersham Pharmacia Biotech, Piscataway, NJ). These constructs allow controlled expression of recombinant 85P1B3 protein sequences with GST fused at the amino-terminus and a six histidine epitope (6X His) (SEQ ID NO: 709) at the carboxyl-terminus. The GST and 6X His (SEQ ID NO: 709) tags permit purification of the recombinant fusion protein from induced bacteria with the appropriate affinity matrix and allow recognition of the fusion protein with anti-GST and anti-His antibodies. The 6X His tag (SEQ ID NO: 709) is generated by adding 6 histidine codons to the cloning primer at the 3' end, e.g., of the open reading frame (ORF). A proteolytic cleavage site, such as the PreScission™ recognition site in pGEX-6P-1, may be employed such that it permits cleavage of the GST tag from 85P1B3-related protein. The ampicillin resistance gene and pBR322 origin permits selection and maintenance of the pGEX plasmids in *E. coli*--

Please replace the paragraph beginning at page 82, line 29, with the following rewritten paragraph:

*A9*

--pMAL Constructs: To generate, in bacteria, recombinant 85P1B3 proteins that are fused to maltose-binding protein (MBP), all or parts of the 85P1B3 cDNA protein coding sequence are fused to the MBP gene by cloning into the pMAL-c2X and pMAL-p2X vectors (New England Biolabs, Beverly, MA). These constructs allow controlled expression of recombinant 85P1B3 protein sequences with MBP fused at the amino-terminus and a 6X His epitope tag (SEQ ID NO: 709) at the carboxyl-terminus. The MBP and 6X His tags (SEQ ID NO: 709) permit purification of the recombinant protein from induced bacteria with the appropriate affinity matrix and allow recognition of the fusion protein with anti-MBP and anti-His antibodies. The 6X His epitope tag (SEQ ID NO: 709) is generated by adding 6 histidine codons to the 3' cloning primer. A Factor Xa recognition site permits cleavage of the pMAL tag from 85P1B3. The pMAL-c2X and pMAL-p2X vectors are optimized to express the recombinant protein in the cytoplasm or periplasm respectively. Periplasm expression enhances folding of proteins with disulfide bonds.--

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Please replace the paragraph beginning at page 83, line 6, with the following rewritten paragraph:

*A10*

--pET Constructs: To express 85P1B3 in bacterial cells, all or parts of the 85P1B3 cDNA protein coding sequence are cloned into the pET family of vectors (Novagen, Madison, WI). These vectors allow tightly controlled expression of recombinant 85P1B3 protein in bacteria with and without fusion to proteins that enhance solubility, such as NusA and thioredoxin (Trx), and epitope tags, such as 6X His (SEQ ID NO: 709) and S-Tag™ that aid purification and detection of the recombinant protein. For example, constructs are made utilizing pET NusA fusion system 43.1 such that regions of the 85P1B3 protein are expressed as amino-terminal fusions to NusA.--

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Please replace the paragraph beginning at page 84, line 4, with the following rewritten paragraph:

*A 11*

--pcDNA4/HisMax Constructs: To express 85P1B3 in mammalian cells, the 85P1B3 ORF, or portions thereof, of 85P1B3 are cloned into pcDNA4/HisMax Version A (Invitrogen, Carlsbad, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter and the SP16 translational enhancer. The recombinant protein has Xpress™ and six histidine (6X His)

*A11*  
*CON'T.*

(SEQ ID NO: 709) epitopes fused to the amino-terminus. The pcDNA4/HisMax vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability along with the SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Zeocin resistance gene allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in *E. coli*.--

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Please replace the paragraph beginning at page 84, line 13, with the following rewritten paragraph:

*A12*

**--pcDNA3.1/MycHis Constructs:** To express 85P1B3 in mammalian cells, the 85P1B3 ORF, or portions thereof, of 85P1B3 with a consensus Kozak translation initiation site are cloned into pcDNA3.1/MycHis Version A (Invitrogen, Carlsbad, CA). Protein expression is driven from the cytomegalovirus (CMV) promoter. The recombinant proteins have the myc epitope and 6X His epitope (SEQ ID NO: 709) fused to the carboxyl-terminus. The pcDNA3.1/MycHis vector also contains the bovine growth hormone (BGH) polyadenylation signal and transcription termination sequence to enhance mRNA stability, along with the SV40 origin for episomal replication and simple vector rescue in cell lines expressing the large T antigen. The Neomycin resistance gene can be used, as it allows for selection of mammalian cells expressing the protein and the ampicillin resistance gene and ColE1 origin permits selection and maintenance of the plasmid in *E. coli*.--

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Please replace the paragraph beginning at page 85, line 1, with the following rewritten paragraph:

*A13*

**--PAPtag:** The 85P1B3 ORF, or portions thereof, of 85P1B3 are cloned into pAPtag-5 (GenHunter Corp. Nashville, TN). This construct generates an alkaline phosphatase fusion at the carboxyl-terminus of the 85P1B3 proteins while fusing the IgG $\kappa$  signal sequence to the amino-terminus. Constructs are also generated in which alkaline phosphatase with an amino-terminal IgG $\kappa$  signal sequence is fused to the amino-terminus of 85P1B3 proteins. The resulting recombinant 85P1B3 proteins are optimized for secretion into the media of transfected mammalian cells and can be used to identify proteins such as ligands or receptors that interact with the 85P1B3 proteins. Protein expression is driven from the CMV promoter and the

*A13*  
*CONT.*

recombinant proteins also contain myc and 6X His epitopes (SEQ ID NO: 709) fused at the carboxyl-terminus that facilitates detection and purification. The Zeocin resistance gene present in the vector allows for selection of mammalian cells expressing the recombinant protein and the ampicillin resistance gene permits selection of the plasmid in *E. coli*.--

Please replace the paragraph beginning at page 85, line 12, with the following rewritten paragraph:

*A14*

--pTag5: The 85P1B3 ORF, or portions thereof, of 85P1B3 was cloned into pTag-5. This vector is similar to pAPtag but without the alkaline phosphatase fusion. This construct generated 85P1B3 protein with an amino-terminal IgG $\kappa$  signal sequence and myc and 6X His epitope (SEQ ID NO: 709) tags at the carboxyl-terminus that facilitate detection and affinity purification. The resulting recombinant 85P1B3 protein was optimized for secretion into the media of transfected mammalian cells, and was used as immunogen or ligand to identify proteins such as ligands or receptors that interact with the 85P1B3 proteins. Protein expression is driven from the CMV promoter. The Zeocin resistance gene present in the vector allows for selection of mammalian cells expressing the protein, and the ampicillin resistance gene permits selection of the plasmid in *E. coli*.--

Please replace the paragraph beginning at page 86, line 10, with the following rewritten paragraph:

*A15*

--Additional pSR $\alpha$  constructs are made that fuse an epitope tag such as the FLAG<sup>TM</sup> tag to the carboxyl-terminus of 85P1B3 sequences to allow detection using anti-Flag antibodies. For example, the FLAG<sup>TM</sup> sequence 5' gat tac aag gat gac gac gat aag 3' (SEQ ID NO: 734) is added to cloning primer at the 3' end of the ORF. Additional pSR $\alpha$  constructs are made to produce both amino-terminal and carboxyl-terminal GFP and myc/6X His (SEQ ID NO: 709) fusion proteins of the full-length 85P1B3 proteins.--

Please replace the paragraph beginning at page 123, line 26, with the following rewritten paragraph:

**--Table XXIA. Nucleotide sequence of splice variant 1 (SEQ ID NO: 701).**

1 TTTTTTTTTT CCTATCTAGC TATCTCTAA AACAAAAGC CATAGTAAAT GCATCAGAGA  
61 TGGATATTCA AAATGTTCCCT CTATCAGAAA AGATTGCAGA GGTAAAATTT CATGATGGTT  
121 GTATGCTTTT TTAAAATACA GACAACTCTT GATAACTTCT ACCAATGAAC TTGGGGATGA  
181 TGAAATGGCA TGATGCTCAA TAATCCTTT TACCTGATTT GACCTTCCT ATTGAATTG  
241 TAATGAAAAA CAAAATACTA AAACCACACT GTAAGGTATA GTTCAGGAAG AAAGGAAAAG  
301 CTGCTCAACT GCTGCACTCC TGCATTCTCC TTTGTGCTGG GAATGGATAT CATCATCTTG  
361 CCATAGAGGT GTCTTCTTTG CAAATACCTT GTAATTGCTC AACTGTCTCA GACATAAGAG  
421 TGATGAAACA GTTATTAAAGA ATTCCTGGCC GGGCGTGGTG GCTCACGCCT GTAATCCCAG  
481 CACTTGGCC TCGTGC

**A16**  
**Table XXIIA. Nucleotide sequence alignment of 85P1B3 with splice variant 1.**

Score = 160 bits (83), Expect = 3e-36

Identities = 83/83 (100%)

Strand = Plus / Plus

85P1B3: 524 gctatctctaaaaacaaaagccatagtaaatgcatacagagatggatattcaaaatgttc 583  
||||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||  
gctatctctaaaaacaaaagccatagtaaatgcatacagagatggatattcaaaatgttc 78  
Vrnt 1: 19

85P1B3: 584 ctctatcagaaaagattgcagag 606 (SEQ ID NO: 702)  
||||||| ||||| |||||  
Vrnt 1: 79 ctctatcagaaaagattgcagag 101 (SEQ ID NO: 703)

**Table XXIIIA. Amino acid sequence alignment of 85P1B3 and splice variant 1.**

Score = 64.8 bits (135), Expect = 2e-08

Identities = 28/29 (96%)

Frame = +1 / +3

85P1B3: 526 YLLKTKAIVNASEMDIQNVPLSEKIAELK 612 (SEQ ID NO: 704)  
YLLKTKAIVNASEMDIQNVPLSEKIAE+K  
Vrnt 1: 21 YLLKTKAIVNASEMDIQNVPLSEKIAEVK 107 (piece of SEQ ID NO: 707) --

Please replace the paragraph beginning at page 124, line 25, with the following rewritten paragraph:

--Table XXIVA. Peptide sequences from the translation of the nucleotide sequence of splice variant 1.

Open reading frame	Amino acid sequences
Frame 1 (SEQ ID NO: 705)	FFF SYLAIS *KQKP**MHQRWIFKMFYQKRLQR*NFM MVVCFKIQTTLDNFYQ*TWG* *NGMMLNNPFYLI*PSLLNL**KTKY*NHTVRYSSGRKEKLLNCCTPAFSFVLGMDIIIL P*RCLLCKYLVIAQLSQT*E**NSY*EFLAGRGGSRL*SQHFGLV
Frame 2 (SEQ ID NO: 706)	FFFPI*LSLKNKSHSKCIRDGYSKCSSIRKDCRGKIS*WLYAFLKYRQLLITSTNELGDD EMA*CSIILFT*FDLPY*ICNEKQNTKTTL*GIVQEERKSCSTAALLHSPLCWESISSC HRGVFFANTL*LLNCLRHKSDTEVIKNSWPGVVVAHACNPSTLASC
Frame 3 (SEQ ID NO: 707)	FFFLSSYLLKTKAIVNASEMDIQNVPLSEKIAEVKFHDGCMLP*NTDNS**LLPMNLGMM KWHDAQ*SFLDDLTFPIEFVMKMKILKPHCKV*FRKKGKAAQLLHSCILLCAGNGYHHILA IEVSSLQIPCNCTVSDIRVMQQLLIPGRAWWLTPVIPALWPR

Note: Frame 3 gives the longest subsequence that is identical with 85P1B3 amino acid sequence. In this Table each (\*) indicates the product of a single codon, i.e., a single unknown amino acid or a stop codon.--

Please replace the paragraph beginning at page 167, line 1, with the following rewritten paragraph:

--Table XIX: Motifs and Post-translational modifications

N-glycosylation site

181-184 NASE (SEQ ID NO: 735)

Protein kinase C phosphorylation site

Number of matches: 4

- 1 24-26 TER
- 2 126-128 SLK
- 3 166-168 SDK
- 4 193-195 SEK

Casein kinase II phosphorylation site

Number of matches: 3

- 1 35-38 TSME (SEQ ID NO: 736)
- 2 183-186 SEMD (SEQ ID NO: 737)
- 3 225-228 SKPE (SEQ ID NO: 738)

N-myristoylation site

Number of matches: 5

- 1 23-28 GTERAI (SEQ ID NO: 739)
- 2 122-127 GIE GSL (SEQ ID NO: 740)
- 3 125-130 GSL KGS (SEQ ID NO: 741)
- 4 129-134 GST YNL (SEQ ID NO: 742)
- 5 141-146 GIP VGF (SEQ ID NO: 743)

RGD Cell attachment sequence

17-19 RGD